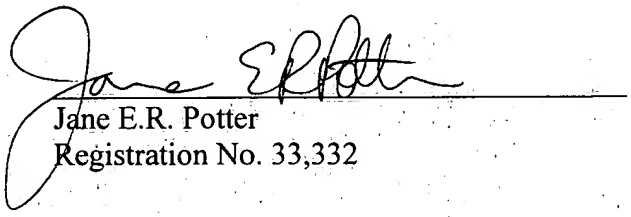
for the A2N mutation were 5' CAG GAA ACA GAC CAT GAA CAT TAA ATT TGC TGG (SEQ ID NO: 31) with its complement and for F39W 5' CAA ACA GCA GAA TGG AAA GGA ACA TTT GAA GAA GC (SEQ ID NO: 32) and its complement. The mutagenesis reactions all contained 125ng of each primer, 0.5 mM dNTPs (0.125mM each dNTP), 1x reaction buffer (10x buffer contains 100mM KCl, 60mM $(NH_4)_2SO_4$, 200mM Tris-HCl (pH 8), 20mM $MgCl_2$, 1% Triton X-100, 100μg/ml nuclease free BSA). The amount of dsDNA template was varied from 5 to 50 ng per reaction and 2.5U cloned *Pfu* polymerase (Stratagene) was added to each. After overlaying with mineral oil the reactions were initially heated to 95°C for 5 minutes, followed by 16 cycles of 95°C for 30 seconds, 58°C for 1 minute and 68°C for 12 minutes. Dpn I enzyme (10 units) was then added to each reaction and, after thorough mixing, the reactions were incubated at 37°C for 4 hours. 2μl from each reaction was then used to transform competent XL1-BLUE *E. Coli* bacteria which were then grown on LB-ampicillin plates. Colonies were picked and DNA prepared. Sequencing was used to check for the presence of the desired mutation.

## REMARKS

The enclosed electronic and paper copies of the Sequence Listing include no new matter that goes beyond the original application as filed, but are supplied to fulfill the requirements as outlined in the Notice to File Missing Parts. Furthermore, the above amendments, which merely direct the insertion of the Sequence Listing and insertion of sequence identifiers, include no matter that goes beyond the original application as filed. Applicant respectfully submits that the above-identified application is now in compliance with 37 C.F.R. §§ 1.821-1.825.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The first of the attached pages is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,
Seed Intellectual Property Law Group PLLC



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Application No. : 09/808,212
Docket No. : 100084.414US

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 14, line 18, has been amended as follows:

The cloning, expression and purification of PpL is described in Bottomley *et al,* Bioseparation, 1995, 5, 359-367. PpL mutants were produced by site-directed mutagenesis and subsequent expression of the mutated PpL gene. Site-directed mutagenesis was carried out using the Kunkel method (Kunkel *et al,* Methods in Enzymol 1987, 154, 367-382). The oligonucleotides used to generate mutations at specific positions were:

-Y64W (substitution of the tyrosine residue at amino acid position 64 by tryptophan):

5' TAAGTCTGCTGTCCATTCGCCATTTAC-3' (SEQ ID NO: 23);

F39H: 5'- TGTTCCTTTATGTTCTGCTGT-3' (SEQ ID NO: 24);

Y53F: 5'- TAATAAGTCTGCGTTTCTGTAAGCTTC-3' (SEQ ID NO: 25);

Y53H: 5'- TAAGTCTGCATGTCTGTAAGC-3' (SEQ ID NO; 26);

L57D: 5'- ATTTACTTTTGCGTCTAAGTCTGCATA-3' (SEQ ID NO: 27);

L57H: 5' TACTTTTGCATGTAAGTCTGC-3' (SEQ ID NO: 28);

59G60 (G inserted between positions 59 and 60):

5'- TTCGCCATTTACACCTTTTGCTAATAAGTC-3' (SEQ ID NO: 29)

N76D: 5'- AAATTTAATGTCCATATGGTT-3' (SEQ ID NO: 30).

Paragraph beginning at page 23, line 21, has been amended as follows:

The double domain PpL gene was mutated using a PCR mutagenesis method. Two primers were designed that annealed to the same sequence on opposite strands of the plasmid and contained the desired mutation close to the middle. The primers were 30-45 bp in length with a melting temperature around 80° C. The primers also had a minimum GC content of 40%, terminated in a G or C, and were HPLC purified. The primers (with the mutations shown in bold) for the A2N mutation were 5' CAG GAA ACA GAC CAT GAA CAT TAA ATT TGC TGG (SEQ ID NO: 31) with its complement and for F39W 5' CAA ACA GCA GAA TGG AAA GGA ACA TTT GAA GAA GC (SEQ ID NO: 32) and its complement. The mutagenesis reactions all contained 125ng of each primer, 0.5 mM dNTPs (0.125mM each dNTP), 1x reaction buffer (10x buffer contains 100mM KCl, 60mM $(NH_4)_2SO_4$, 200mM Tris-HCl (pH 8), 20mM $MgCl_2$, 1% Triton X-100, 100μg/ml nuclease free BSA). The amount of dsDNA template was varied from 5 to 50 ng per reaction and 2.5U cloned *Pfu* polymerase (Stratagene) was added to each. After overlaying with mineral oil the reactions were initially heated to 95°C for 5 minutes, followed by 16 cycles of 95°C for 30 seconds, 58°C for 1 minute and 68°C for 12 minutes. Dpn I enzyme (10 units) was then added to each reaction and, after thorough mixing, the reactions were incubated at 37°C for 4 hours. 2μl from each reaction was then used to transform competent XL1-BLUE *E. Coli* bacteria which were then grown on LB-ampicillin plates. Colonies were picked and DNA prepared. Sequencing was used to check for the presence of the desired mutation.